decreased expression of the matrix metalloproteinase MMP13, the major collagen type II degrading enzyme in osteoarthritis.
Conclusion: Our results demonstrate that expression of the collagen binding integrin a10b1 on chondrocytes regulates expression of cartilage specific molecules as well as the collagen type II degrading enzyme MMP13. This indicate that a10b1 may play an important role in modulating the critical balance between synthesis and degradation of the cartilage matrix.

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PROTEOMICS STUDY OF CARTILAGE BREAKDOWN CAUSED BY MECHANICAL INJURY AND CYTOKINES IL-1β AND TNF-α

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Objective: The aim of this study is to use a systems-level mass spectrometry based proteomics approach to examine and to quantify the individual and synergistic actions of injurious mechanical compression of cartilage and treatment with TNF-α and IL-1β in an *in vitro* model of joint injury, with the goal of understanding potential mechanistic pathways that lead to cartilage degradation.
Methods: Cartilage explants were harvested from the femoropatellar groove of bovine calves. 5 or 7 days post harvesting, explants were treated with a single uniaxial, radially unconfined compression to 50% strain at 100%/sec, IL-1β (10ng/ml), TNF-α (100 ng/ml), or untreated. For all studies, 10% of medium was changed daily until treatment was ended at day 5 after injury/cytokine. The collected, pooled medium was dialyzed with chondroitinase ABC to remove sGAG, concentrated, and subjected to SDS-PAGE. Each lane was cut into ~30 slices that were in-gel digested and subjected to reverse phase nano-LC/MS/MS. Using a Vydac C18 column peptides were eluted using a 120 minute gradient of 3-80% acetonitrile (1.2% acetic acid) on to an ABI QStar mass spectrometer. The data were analyzed using Spectrum Mill software searching against NCBInr bovine and mammalian protein databases. Proteins were identified by greater than two peptides. Because we wanted to identify proteins which were distinctly associated with particular treatments, we used a K-means clustering algorithm to group proteins according to their presence in the four conditions tested using number of spectra as an indicator of protein quantity. As these are semi-quantitative analyses, we report only large differences, which likely reflect significant changes.
Results: We have identified approximately 275 proteins present in the medium of the untreated, IL-1β treated, TNF-α treated, and the injuriously compressed samples. Clustering analyses revealed the release of heat shock and chaperone proteins in response to mechanical injury, and the release of several annexin family members (1, A2, 5, A8) in response to TNF-α treatment. Heat shock/chaperone proteins released in response to injury include GRP78, PDI, Hsp90, gp96, Txndc7, calreticulin, and Hsp70 protein 8. Additionally, IL-1β caused the release of proteins such as MMP-3, serum amyloid A3, CD14, lactoferrin, and chitinase-3-like-2, which are known to be associated with cartilage breakdown and inflammatory response. Other identified proteins included ECM structural proteins, proteases, cartilage growth regulatory pathways, angiogenic regulation, and inflammatory response. ECM proteins including aggrecan, link protein, COMP, matrilin-1, thrombospondin-1, and fibronectin were present in low MW fractions indicating degradation.
Conclusion: Protein profiling together with clustering analyses revealed a number of distinguishing features of each treatment that may serve as markers for particular types of degradative processes. We are currently using isotope labeling to further quantify and to verify the differences between treatments to highlight differences and possible synergisms between mechanical injury and inflammatory cytokines.

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CARTILAGE GENES AND IMPACT INJURY

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Our goal was to identify several genes which have a previously undescribed role in the pathogenesis of OA. We used mechanical impact on cartilage *in vitro* to mimic osteoarthritic parameters, including cellular and matrix responses, in order to study early events leading to cartilage degeneration. Microarray experiments using the Affymetrix Canine GeneChip, which can measure expression of 23,836 genes simultaneously, identified 32 genes as significantly differentially expressed when the false discovery rate was held to 10%. We used quantitative reverse transcription polymerase chain reaction (q RT-PCR) to confirm the microarray data. To understand how genes which respond to mechanical impact could have a role in cartilage degeneration *in vivo,* expression of each gene in the degenerated cartilage of an OA lesion (LES) from three dogs with hip OA was compared with expression in site-matched cartilage (LA) from three normal dogs and with expression in unaffected surrounding cartilage (SA). We report for the first time that mRNA expression of dynein cytoplasmic light polypeptide, a component of intracellular motors, was increased in response to impact load. mRNA levels of dynein were also higher in lesions than in healthy cartilage. Results were compared to two known cartilage genes, fibronectin and MMP 13 (collagenase 3), and to a novel cartilage gene, MIG-6/Gene 33, for which we recently published data.

Changes in mRNA levels in impact damaged cartilage

Gene	Control	Load	Fold Change
Fibronectin	33.6±0.96	84.9±0.97	2.5
MMP 13	4.6±0.27	4.2±0.44	0.9
Dynein	9.6±0.29	23.4±1.6	2.4
MIG-6	0.9±0.23	3.4±0.15	3.8

mRNA levels for each gene were determined by q RT-PCR, normalized for beta actin and expressed as femtomoles/ng RNA ± SD (n=3).

Changes in mRNA levels in cartilage with an OA lesion

Gene	Normal Dog		OA Dog		Fold Change	
	SA	LA	SA	LES	LES/SA-OA	LES/LA
Fibronectin	66.7±3.1	115±5.1	90.4±10.1	219±7.2	2.4	1.9
MMP 13	4.4±0.03	4.9±0.13	4.3±0.06	4.1±0.55	0.95	0.84
Dynein	0.82±0.06	0.99±0.16	0.96±0.18	3.6±0.51	3.75	3.6
MIG-6	1.3±0.2	0.8±0.29	1.7±0.64	3.6±1.2	2.1	4.5

mRNA levels for each gene were determined by q RT-PCR, normalized for beta actin and expressed as femtomoles/ng RNA ± SD (n=3).

The q RT-PCR data confirmed up-regulation in response to load for dynein as well as for MIG-6 and confirmed no response for MMP 13. The increase in fibronectin RNA levels in response to load and in lesion cartilage was consistent with previous data from our laboratory on the protein level. We have observed intense but localized staining of active MMP 13 in cartilage lesions which mRNA levels from full depth sections failed to reflect. Data suggest that MIG-6, which is involved in establishing chronic response to stress in other tissues, and dynein cytoplasmic light chain, which sequesters pro-apoptotic proteins, respond to impact and may be important for initiation of cartilage degeneration.